



Effect of AM fungal association on the growth performance of selected medicinal herbs

KEYWORDS

Medicinal weeds, AMF, symbiosis, growth performance, enhanced biomass

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ABSTRACT Effect of various AM fungal species (*Acaulospora birticulata*, *A.lacunosa*, *A.laevis*, *Glomus aggregatum*, *G.fasciculatum*, *G.geosporum* and *G.mosseae*) on the growth performance of commonly grown medicinal weeds such as *Achyranthes aspera*, *Eclipta alba*, *Euphorbia hirta* and *Leucas aspera* was studied. Percent germination of seeds was significantly enhanced in presence of AM fungi. Rhizosphere samples collected at the time of seed collection in their natural habitats and after 90 days of AMF inoculation revealed the abundance of spores of *Glomus* species. Among the AM fungi used in the present study, *G.fasciculatum* showed maximum colonization with 100% in all the selected plants. The correlation between the percent colonization and the biomass was not observed in the present study. The root and shoot length along with biomass in all the selected plants varied with the AMF species indicating the specific host compatibility. AMF inoculated plants in general, showed better growth performance than control plants.

INTRODUCTION

India is regarded as the treasure house of herbs in the world. Herbal medicines are still the main stream of the majority of the world population, mainly in the developing countries for primary healthcare because of better cultural acceptability, compatibility with in human body and lesser side effects. Many herbal medicinal plants are often treated as weeds because of their undesirable habitats. However, the fact remains that they are often very powerful medicinal plants having high potential to address many of today's major health problems.

AM fungi are known to improve the plant growth through better uptake of water and the nutrients, particularly Phosphorus (Lambert *et al.* 1979 and Brundrett, 2009). The symbiotic association also improves the host resistance to drought and pathogens (Kothamati *et al.* 2001). Thus improved physiological status is reflected in their better growth performance. The symbiotic association not only enhances the growth performance of the host plants but also improves the production of active principles (Sowmya *et al.* 2004 and Tejavathi *et al.* 2011). The enhanced biomass and active principles in the AM fungal associated medicinal plants can be harnessed by pharmaceutical industries. Standardization of optimum conditions for the better growth of the medicinal herbs with enhanced active principles is of utmost important in the commercialization of these taxa. Hence, the present studies deals with the effect of AM fungi on the growth performance of the selected medicinal plants and feasibility of utilizing this strategy for their systematic cultivation.

MATERIAL AND METHODS

Achyranthes aspera L., *Eclipta alba* (L.) Hassk., *Euphorbia hirta* L. and *Leucas aspera* (Willd.)Sperg which are commonly grown as weeds all along the roadside, gutters and open fields were selected for the present study. Mature seeds were collected from the plants by frequent visits to their habitats in and around Bangalore, Karnataka. They were subjected to seed health and seed viability tests following the guidelines of ISTA (1996).

The AM fungal species selected for the present study were *Acaulospora birticulata*, *A.lacunosa*, *A.laevis*, *Glomus aggregatum*, *G.fasciculatum*, *G.geosporum* and *G.mosseae* and the inoculums were obtained from Department of Agricultural Microbiology, University of Agricultural Sciences,

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Seed health and viability test

Paper towel and Blotter methods were followed in order to test the seed health and viability. Both dry and pre soaked seeds in water for overnight were used for this study.

Paper towel method: This method is a routine procedure for seed health testing of suspected seed lot. Two papers towels/germinating papers measuring 45x28 cm were soaked in distilled water. 100 presoaked and dry seeds of each selected taxon were randomly selected and placed on wet paper towel with equally spaced 10 seeds in a row. The other paper was used for covering the seeds. These towels were rolled and ends were tied with rubber bands and placed in an upright or inclined in a plastic tray. The whole setup was incubated for 10 days at 27-30°C under 12 h light and dark regime. After 10th day, the paper towels were unrolled and percent of infection and germination were recorded.

Blotter method: 100 seeds of dry and presoaked of each selected species were plated on well soaked filter paper with sterile distilled water in groups of 25 per petriplates. Each plating was facilitated by rotating the petriplate slowly and smoothly in clockwise manner with 15 seeds in the outer ring, 9 in the middle and one in the centre at equidistance. They were incubated for 10 days at 27-30°C under 12:12 light and dark regime. After 10th day, the seeds were examined for microbial infection and percent germination.

Study of soil samples from Rhizosphere.

Soil samples were collected from rhizospheres of selected medicinal plants at the time of seed collection. They were analyzed for mycorrhizal spore count by wet sieving and decanting technique of Gerdemann and Nicolson (1963).

Soil samples from the rhizospheres of all the selected plants after 90 days of AM fungal inoculation were analyzed for mycorrhizal spore count by following the method of Gerdemann and Nicolson (1963).

Green house test

Green house test was carried out to determine the compatibility between AMF load and selected plant species. The experiment was designed with 8 treatments in 4 replicates. Totally 96 uniformly sized plastic pots (7x7") capacity of holding

4 kg of sterilized sandy loam soil (autoclaved twice at 108 kpa for 15 min) were set up at green house in the department of Botany, Bangalore University. pH of the soil was adjust to 6.8 to 7. AMF inoculum of 0.8g (1gm = 1 lakh propagules) was placed at the depth of 2cm from the soil surface. The pre-soaked mature seeds collected from the selected plants were sterilized with 1% sodium hypochlorite were placed over the surface of the sterilized soil, pushed down to 1.5 cms deep in the soil and covered by the surrounding soil. Regular watering with shower was done on every alternate day and maintained for 90 days under green house conditions. The seedlings were thinned after 12 days of emergence to maintain 2 seedlings per pot. Pots without AMF inoculums served as control. Root and shoot length along with biomass were recorded after 30, 60 and 90 days of AM inoculation and thus obtained data was recorded and tabulated.

AM fungal colonization

The AMF treated medicinal plants were carefully uprooted after 30, 60 and 90 days of inoculation and thoroughly washed under running tap water. Fresh root samples were cut into 1 cm segments and placed in plastic vials containing 10 ml of 10% KOH for 48h at room temperature (Phillips and Haymann, 1970). After decanting the alkali, the residual alkali was neutralized by immersing them in 10ml of 10% HCl for 15 min. After through washing in double distilled water, the root segments were stained with 0.1% Trypan blue in lactoglycerol for 24 h.

Percent of AM fungal colonization was calculated using the following formula

$$\% \text{ Colonization} = \frac{\text{No. of segments colonized with AM}}{\text{Total no. of segments}} \times 100$$

Data analysis

The data thus obtained from the experiments were subjected to one-way analysis of variance (ANOVA) for completely randomized design (CRD) using MSTAT-C software. The treatment means were separated by Duncan's Multiple Range Test (DMRT) at 5% level of significance (Little and Hills, 1978).

RESULTS AND DISCUSSION

For the establishment of a good crop, uniform and maximum germination of seeds and emergence of strong and sturdy seedlings are necessary as they are able to survive under the unfavorable conditions. The seed samples of the selected medicinal plants were collected from in and around Bangalore and subjected to Blotter and Paper towel methods to analyze the seed health, viability and percent germination. Presoaked seeds showed better percent of germination than dry seeds, because of the high metabolic activity due to prior water imbibitions. The percent germination in blotter method was significantly higher than the paper roll method, since many seeds were damaged in the process of rolling. Hence the results from the blotter method were recorded. Highest percent of germination was recorded in *E.hirta* with 85% and lowest of 48% in *L.aspera*. Seed health testing is critical for insuring the health of basic seed stocks used for seed production. It is also an essential management tool for the control of seed borne and seed transmitted pathogens (Morrison, 1999).

The seeds of all selected plants in control have germinated after 10 days of incubation in the soil. Presence of AMF in the soil, however, enhanced the percent germination and also reduces the time taken for the germination. About 90% of germination was recorded in the seeds of all the selected species when they were sown in the soil mixed with inoculums of various AM fungi. Time taken for germination and percent germination varies with the AM fungi present in the soil. Seeds of *E. hirta* have germinated within 2 days of sowing in presence of *G.mosseae*, whereas in other species of AMF, they have taken 4-5 days for germination. Similarly the time taken for germination of seeds of *E.alba*, *A.aspera* and *L.aspera*

was reduced to 5 days in the presence of *A.bireticulata*, *A.lacunosa*, *G. aggregatum*, *G.fasciculatum* and *G.mosseae*, whereas 7 days in *A.laevis* and *G.geosporum*. The enhanced percent of germination in all the selected species in presence of fungi may be attributed to the secretion of phytohormones by AM fungi (Azcon-aguilar and Barea, 1978). However, Jala-luddin and Hamid (2011) did not find the promotory effect of AM fungi on germination in the varieties of sunflower.

Soil samples were collected from the rhizospheres of all the selected medicinal herbs during seed harvesting. Presence of AM spores in the samples varies with the species. A few spores of AMF were identified in the rhizosphere samples of *E.alba* and *E.hirta*. However, the rhizosphere sample of *A.aspera* revealed many AMF spores, while no AMF spores were identified in the rhizosphere of *L.aspera*. The AMF spores found in the rhizosphere were identified by using the manual for identification (Schenck and Perez, 1990). The most common AMF spores that were found in the rhizosphere samples were belong to *Glomus*, *Acaulospora* and *Gigapora*. Among these, *Glomus* and *Acaulospora* spores were more abundant in the samples. *Glomus* in the rhizospheres of medicinal plants was reported in a several earlier studies (Allen et al. 1995, Francis and Read, 1994 and Selvaraj et al. 2001). The main reason for their abundance may be their ability to sustain wide range of temperatures and pH for spore germination (Wang et al. 1997). Whereas the spores of *Acaulospora*, which are found in abundance in the rhizosphere samples of *A.aspera* are often associated with acidic soils (Abbott and Robson, 1991). Allen et al. (1995) and Francis and Read (1994) have reported high AM fungal diversity in the rhizosphere of medicinal plants.

Since the spores of *Glomus* and *Acaulospora* were seen in the rhizosphere of these plants in their natural habitats, they were selected for the present study. Root segments of all the selected plants exhibited colonization by arbuscular mycorrhizal fungi. Among the various AMF species used in the present study, *G.fasciculatum* was found to be more compatible with all the four selected medicinal herbs with 100% colonization. Next best was found to be *G.mosseae* with 100% colonization in *E.hirta*, *E.alba* and *L.aspera*. However it showed only 30% of colonization in *A.aspera*. Compatible association of *G.fasciculatum* with other medicinal plants is well documented (Bhagyaraj and Manjunath, 1980, Rao et al. 1989, Karthikeyan et al. 2009 and Tejavathi et al. 2011). At the time of harvest, the root and shoot biomass of the inoculated plants was found to be maximum and significant than that of control in all the four selected plants. *G.mosseae* was found to be exerting profound influence on the growth of *E. hirta* and *L.aspera* with total fresh biomass being recorded as 4.96 ± 0.12 and 13.18 ± 0.35 (Table 3 & 4) respectively, whereas *G.aggregatum* and *G.fasciculatum* enhanced the growth performance of *E.alba* and *A.aspera* with fresh total biomass of 9.99 ± 0.59 and 11.92 ± 0.55 (Table 1 & 2) respectively. These observations have substantiated the hypothesis that not all the combinations of host and endophytes have similar growth stimulating effects (Hayman, 1980). This may be due to the specific host compatibility as reported in other plants (Sharada Khade and Rodrigues, 2009). The extent of improvement by AM fungi varied with the species of AM fungi and may be depends on their capacity to take up P from soil and transfer into the host. Host specificity of AM fungi is also well documented by Smith et al. (2000) and Burleigh et al. (2002). Hence selection of efficient AM fungi plays a significant role in achieving maximum benefits from the association. Similar improved growth and higher biomass in medicinal plants were earlier reported by Kumar and Muruges (2002), Karthikeyan (2009) and Tejavathi et al. (2011).

In the present study, 100% colonization of *G.fasciculatum* was recorded in all the four selected species, but it was more efficient in enhancing the growth performance in *A. aspera* though it substantially improved the growth performance in other species. Further, as Yosef et al. (1984) and Declerck

et al. (1989) pointed out that the amount of colonization by AMF is not always correlated with their efficiency in improving the growth of the host plants. However, Sharada Khade and Rodrigues (2009) have reported correlation between the root colonization and the efficiency of *G.mosseae* in increasing the growth parameters in Papaya. Whereas, Arpana and Bagyaraj (2007) have not reported correlation between the percent colonization with total biomass in *Andrographis paniculata* which is in accordance with our study. In the present study, higher sporulation by specific fungi in all the selected plants correlated with the higher biomass. While, in *Andrographis paniculata*, the extent of root colonization and the spore count varied with different AM fungi (Chiramel et al. 2006).

Conclusion

AM fungi association in general has enhanced the growth

performance of all the selected medicinal herbs compared to control plants. The maximum benefit on enhancement and biomass depends on the compatible specific AM fungal association. Since the whole plant is used in indigenous systems of medicine to cure the ailments, increase in biomass will definitely have a significant value. Better still, farming with AM fungi of such medicinally important herbs should be encouraged and promoted as they are actually becoming rare due to urbanization and the harvesting of these species should at the very least be regulated.

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Table 1: Percent Root Colonization, Spore Count of different AM fungi and Biomass in *A.aspera*(90days after inoculation)

Treatments	%Root Colonization	No.Spore / 10g	Fresh weight			Dry weight		
			Root	Shoot	whole plant	Root	Shoot	whole plant
Control	1.20 ^a ± 1.79	0.00 ^a ± 0.00	0.97 ^e ± 0.00	2.84 ^f ± 0.70	3.80 ^f ± 0.70	0.39 ^d ± 0.05	1.33 ^e ± 0.22	1.72 ^e ± 0.21
A.bireticulata	73.40 ^c ± 3.29	41.60 ^c ± 2.61	2.44 ^d ± 0.53	4.16 ^e ± 0.55	7.00 ^e ± 1.00	1.10 ^{bc} ± 0.18	2.18 ^d ± 0.24	3.28 ^d ± 0.35
A.laevis	37.60 ^f ± 2.41	11.40 ^f ± 1.14	2.60 ^{cd} ± 0.35	5.46 ^d ± 0.69	8.06 ^{de} ± 1.03	1.05 ^c ± 0.16	2.29 ^d ± 0.18	3.34 ^d ± 0.26
A.lacunosa	50.20 ^e ± 5.26	16.80 ^e ± 3.35	3.18 ^b ± 0.18	6.43 ^c ± 0.72	9.81 ^{bc} ± 0.84	1.23 ^{abc} ± 0.23	3.22 ^{bc} ± 0.55	4.46 ^{bc} ± 0.75
G..aggregatum	82.00 ^b ± 2.74	41.20 ^c ± 2.77	3.22 ^{ab} ± 0.49	6.38 ^c ± 0.33	9.59 ^{bcd} ± 0.76	1.35 ^{ab} ± 0.04	3.13 ^{bc} ± 0.29	4.48 ^{bc} ± 0.32
G..fasciculatum	100.00 ^a ± 0.00	64.00 ^a ± 5.29	3.49 ^a ± 0.14	8.42 ^a ± 0.52	11.92 ^a ± 0.53	1.49 ^a ± 0.18	4.19 ^a ± 0.29	5.68 ^a ± 0.34
G..geosporum	59.40 ^d ± 4.72	26.00 ^d ± 1.87	2.97 ^c ± 0.51	5.33 ^{de} ± 0.18	8.51 ^d ± 0.73	1.14 ^{bc} ± 0.10	2.73 ^c ± 0.28	3.86 ^c ± 0.24
G..mosseae	98.20 ^a ± 4.02	54.80 ^b ± 4.15	3.26 ^{ab} ± 0.20	7.14 ^b ± 0.48	10.60 ^{ab} ± 0.65	1.25 ^{abc} ± 0.07	3.55 ^b ± 0.22	4.81 ^b ± 0.09

*Means (n=5) in each column followed by the same letter are not significantly different (p<0.05) from each other according to DMRT.

Table 2: Percent Root Colonization and Spore Count of different AM fungi on Biomass in *E.alba* (90days after inoculation)

Treatments	% Root Colonization	No.Spore / 10g	Fresh weight			Dry weight		
			Root	Shoot	whole plant	Root	Shoot	whole plant
Control	1.00 ^e ± 1.00	1.20 ^f ± 1.79	1.27 ^c ± 0.18	3.22 ^e ± 0.09	4.48 ^e ± 0.21	0.63 ^d ± 0.09	1.59 ^c ± 0.03	2.23 ^a ± 0.09
A.bireticulata	100.00 ^a ± 0.00	60.40 ^c ± 5.68	2.19 ^a ± 0.09	4.45 ^d ± 0.03	9.55 ^{ab} ± 0.57	1.10 ^c ± 0.05	2.22 ^{bc} ± 0.02	3.32 ^d ± 0.06
A.laevis	42.20 ^d ± 5.17	28.60 ^a ± 2.30	3.07 ^a ± 0.04	6.32 ^b ± 0.22	6.66 ^d ± 0.08	1.50 ^b ± 0.04	3.37 ^a ± 0.00	4.60 ^b ± 0.28
A.lacunosa	56.80 ^c ± 2.17	31.40 ^d ± 4.22	2.29 ^b ± 0.05	4.54 ^d ± 0.03	6.81 ^c ± 0.08	1.13 ^{bc} ± 0.03	2.25 ^b ± 0.00	3.38 ^d ± 0.03
G..aggregatum	100.00 ^a ± 0.00	83.80 ^a ± 4.87	3.17 ^a ± 0.46	6.81 ^a ± 0.18	9.99 ^a ± 0.59	1.63 ^a ± 0.09	3.44 ^a ± 0.04	5.01 ^a ± 0.25
G..fasciculatum	100.00 ^a ± 0.00	68.40 ^b ± 5.81	2.88 ^a ± 0.33	6.49 ^{ab} ± 0.58	9.20 ^b ± 0.48	1.51 ^{ab} ± 0.04	3.17 ^{ab} ± 0.11	4.61 ^b ± 0.24
G..geosporum	82.40 ^b ± 4.39	38.80 ^d ± 5.63	2.83 ^a ± 0.40	5.82 ^c ± 0.31	8.65 ^{bc} ± 0.44	1.13 ^{bc} ± 0.21	2.86 ^{ab} ± 0.15	4.27 ^c ± 0.19
G..mosseae	100.00 ^a ± 0.00	81.60 ^a ± 6.84	2.96 ^a ± 0.37	6.61 ^{ab} ± 0.10	9.57 ^{ab} ± 0.46	1.54 ^{ab} ± 0.05	3.39 ^a ± 0.00	4.87 ^a ± 0.18

*Means (n=5) in each column followed by the same letter are not significantly different (p<0.05) from each other according to DMRT.

Table 3: Percent Root Colonization and Spore Count of different AM fungi on Biomass in *E.hirta* (90days after inoculation)

Treatments	% Root Colonization	No.Spore / 10g	Fresh weight			Dry weight		
			Root	Shoot	whole plant	Root	Shoot	whole plant
Control	0.80 ^f ± 0.84	0.80 ^d ± 0.84	0.76 ^d ± 0.11	1.09 ^d ± 0.33	1.86 ^e ± 0.25	0.38 ^f ± 0.05	0.55 ^e ± 0.16	0.93 ^d ± 0.13
A.bireticulata	95.20 ^b ± 3.63	45.80 ^b ± 6.98	1.49 ^b ± 0.04	3.03 ^a ± 0.04	4.52 ^b ± 0.05	0.76 ^b ± 0.02	1.12 ^c ± 0.21	2.25 ^a ± 0.02
A.laevis	23.40 ^a ± 1.14	15.20 ^c ± 2.77	1.10 ^c ± 0.06	1.34 ^{cd} ± 0.02	2.44 ^d ± 0.04	0.54 ^{de} ± 0.03	0.68 ^{de} ± 0.03	1.24 ^c ± 0.03
A.lacunosa	33.20 ^d ± 0.84	18.00 ^a ± 2.24	1.13 ^c ± 0.09	1.55 ^c ± 0.02	2.68 ^d ± 0.09	0.53 ^e ± 0.02	0.78 ^d ± 0.01	1.34 ^c ± 0.05
G..aggregatum	100.00 ^a ± 0.00	49.60 ^b ± 14.05	1.39 ^b ± 0.15	3.11 ^a ± 0.08	4.49 ^c ± 0.21	0.70 ^c ± 0.08	1.25 ^c ± 0.27	2.24 ^a ± 0.10
G..fasciculatum	100.00 ^a ± 0.00	60.00 ^a ± 8.15	1.50 ^b ± 0.05	3.14 ^a ± 0.09	4.64 ^b ± 0.12	0.75 ^{bc} ± 0.03	1.53 ^b ± 0.18	2.32 ^a ± 0.06
G..geosporum	76.60 ^c ± 7.40	43.80 ^b ± 6.38	1.13 ^c ± 0.05	2.44 ^b ± 0.43	3.57 ^c ± 0.45	0.59 ^d ± 0.03	1.24 ^c ± 0.09	1.77 ^b ± 0.22
G..mosseae	100.00 ^a ± 0.00	67.40 ^a ± 9.48	1.69 ^a ± 0.07	3.27 ^a ± 0.13	4.96 ^a ± 0.12	0.84 ^a ± 0.03	1.78 ^a ± 0.04	2.38 ^a ± 0.20

*Means (n=5) in each column followed by the same letter are not significantly different (p<0.05) from each other according to DMRT.

Table 4: Percent Root Colonization and Spore Count of different AM fungi on Biomass in *L.aspera* (90days after inoculation)

Treatments	% Root Colonization	No.Spore / 10g	Fresh weight			Dry weight		
			Root	Shoot	whole plant	Root	Shoot	whole plant
Control	2.00 ^f ± 1.58	2.60 ^b ± 1.34	1.86 ^d ± 0.06	3.93 ^e ± 0.04	5.84 ^a ± 0.00	0.94 ^e ± 0.00	1.97 ^e ± 0.01	2.91 ^f ± 0.01
A.bireticulata	90.80 ^b ± 2.95	43.80 ^d ± 4.32	3.45 ^b ± 0.29	5.90 ^c ± 0.98	9.35 ^d ± 1.08	1.72 ^{bc} ± 0.14	3.45 ^{cd} ± 0.28	5.17 ^{cd} ± 0.32
A.laevis	56.20 ^a ± 1.64	18.00 ^a ± 1.73	2.86 ^c ± 0.01	4.61 ^d ± 0.24	7.47 ^f ± 0.23	1.44 ^d ± 0.01	2.50 ^a ± 0.50	3.94 ^e ± 0.50
A.lacunosa	68.00 ^d ± 2.35	24.00 ^f ± 3.67	2.83 ^c ± 0.05	4.58 ^d ± 0.15	7.41 ^f ± 0.14	1.43 ^d ± 0.02	2.29 ^e ± 0.08	3.72 ^{ef} ± 0.09

G..aggregatum	98.00 ^a ± 2.35	51.00 ^c ± 1.41	3.48 ^b ± 0.41	7.87 ^b ± 0.03	11.34 ^c ± 0.43	1.64 ^b ± 0.25	4.00 ^c ± 0.57	5.64 ^c ± 0.66
G..fasciculatum	100.00 ^a ± 0.00	60.80 ^b ± 3.77	3.53 ^{ab} ± 0.42	8.91 ^a ± 0.04	12.44 ^b ± 0.43	1.84 ^{ab} ± 0.02	5.45 ^b ± 0.71	7.29 ^b ± 0.72
G..geosporum	81.00 ^c ± 3.67	30.80 ^e ± 5.17	3.18 ^{bc} ± 0.43	5.08 ^{cd} ± 0.08	8.26 ^e ± 0.51	1.58 ^c ± 0.21	3.31 ^d ± 0.47	4.88 ^d ± 0.67
G..mosseae	100.00 ^a ± 0.00	69.80 ^a ± 9.42	3.86 ^a ± 0.02	9.32 ^a ± 0.37	13.18 ^a ± 0.35	1.92 ^a ± 0.01	7.14 ^a ± 0.58	9.06 ^a ± 0.58

*Means (n=5) in each column followed by the same letter are not significantly different ($p < 0.05$) from each other according to DMRT.

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